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Relevance of Inapparent Coinfection by Hepatitis B Virus in Alpha Interferon-Treated Patients With Hepatitis C Virus Chronic Hepatitis

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The aim of the study was to investigate whether an ''inapparent'' coinfection by hepatitis B virus (HBV) in anti-HCV-positive chronic liver disease patients may influence interferon (IFN) response. Fourteen anti-HCV-positive, hepatitis B surface antigen (HBsAg)-negative but serum HBV-DNA-positive patients and 111 anti-HCV-positive, HBsAg-negative, and HBV-DNA-negative patients with chronic hepatitis were treated with 3 MU of recombinant α-2a IFN 3/week for 1.2 months. Serum HBV-DNA and HCV-RNA were determined before treatment, after 6–12 months, and at the time of alanine aminotransferase (ALT) flare-up by HBV polymerase chain reaction (PCR) and HCV PCR, respectively. IgM anti-HBc were tested using the IMx Core-M assay (Abbott Laboratories, North Chicago, IL). By the end of treatment, ALT values had become normal in 4/14 HBV-DNA-positive patients (28%), but all ''responders'' (4/4) relapsed. IgM anti-HBc was detected both before treatment and during ALT elevation in three patients and only during ALT relapse in another three. In the remaining 111 patients, a biochemical response to IFN treatment was observed in 54% and relapse of ALT values in 47%. ''Inapparent'' HBV/HCV coinfection may be implicated in cases of resistance to IFN. HBV replication and HBV-related liver damage may persist in patients in whom HCV replication was inhibited by current doses of IFN, as suggested also by the presence of IgM anti-HBc in some cases. Further studies will show the effect of different treatment schedules. HBV-DNA and/or IgM anti-HBc detection with very sensitive methods may be important both as a prognostic factor and as a tool for better understanding of inter-virus relationships and mechanisms involved in multiple hepatitis virus infections. ***J. Med. Virol. 51:313–318, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: double infection; HBV; HCV; IFN treatment

INTRODUCTION

Chronic hepatitis C virus (HCV) infection represents a major clinical problem worldwide in that it is responsible for a large number of chronic liver diseases (CLD) [Choo et al., 1990], progressing to cirrhosis and eventually to hepatocellular carcinoma in a significant proportion of cases [Koretz et al., 1985; Ikeda et al., 1993]. Usually, anti-HCV antibody (anti-HCV) detection in patients with CLD, in the absence of hepatitis B virus (HBV) markers as well as different known causes of CLD, supports diagnosis of hepatitis C and its treatment. Although the possibility of HBV infection in hepatitis B surface antigen (HBsAg)-negative subjects has been demonstrated [Zignego et al., 1994], the existence of an "inapparent" coinfection by HBV in HCV-positive, HBsAg-negative patients is not usually investigated in clinical practice [Thiers et al., 1988; Ruiz et al., 1992].

Interferon (IFN) was used as an effective treatment in chronic hepatitis C [Hoofnagle et al., 1987; Di Bisceglie et al., 1989; Alberti et al., 1992]. With currently recommended regimens of therapy, about 50% of patients show normalisation of transaminases and suppression of viral activity during therapy. However, these beneficial effects are often transient and almost half of those whose serum aminotransferase levels became normal with IFN treatment relapse after stopping therapy.

The mechanisms responsible for resistance to treatment and/or relapse of hepatitis after IFN withdrawal have not been clarified, although various viral and/or host factors have been suggested to play a role [Matsumoto et al., 1994, Garson et al., 1995; Suzuki et al., 1995; Ounanian et al., 1995]. A possible factor influencing the responsiveness of HCV-positive patients to IFN treatment may be an "inapparent" coinfection by

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HBV. In fact, higher doses of IFN are needed in patients with chronic HBV infection [Perillo, 1989; Fattovich et al., 1989]. Furthermore, contemporary infection by two different viruses may worsen prognosis and make therapy less effective [Farci et al., 1994; Weltman et al., 1995; Liaw, 1995]. We therefore investigated whether "inapparent" coinfection by HBV in anti-HCV-positive, HBsAg-negative chronic hepatitis patients influences the response to IFN therapy.

PATIENTS AND METHODS

Fourteen patients (13 males and one female; mean age 49.5 ± 8.4 years, range 33–63 years) with chronic active hepatitis (CAH) were studied. At the time of enrollment in the study, all patients were anti-HCV-positive (RIBA II) for more than 1 year and HBsAg- and HBV-DNA-negative using a routine hybridisation technique (Spot test) [Scotto et al., 1983] but positive using polymerase chain reaction (PCR). The first technique in our hands detects levels of viraemia greater than 10^5 HBV DNA copies/ml, while HBV PCR may allow successful amplification with as few as one to ten HBV-DNA molecules in the serum sample [Feray et al., 1990]. The main demographic, clinical, and biological characteristics of the subjects studied are shown in Table I. These patients came from a group of 125 consecutive subjects with chronic anti-HCV-positive, HBsAg-negative hepatitis, recruited over a period of 18 months, who were tested for an inapparent HBV infection by HBV PCR. None of the patients had histological and/or clinical evidence of cirrhosis or a history of antiviral or immunosuppressive treatments, illicit drug use, and/or homosexuality. Furthermore, all patients were negative for anti-HD, HIV, and anti-nuclear/antimitochondrial antibody. The remaining 111 patients (70 males and 41 females) had a mean age of 50.5 ± 11.4 years (range 20–70 years). In all 125 patients (14 HBV-DNA-positive and 111 HBV-DNA-negative), alanine aminotransferase (ALT) values were more than two times normal for at least 6 months and were programmed to receive 3 million units of recombinant IFN α-2a three times a week for 12 months. After IFN withdrawal, patients were followed for at least 12 months.

Written informed consent was obtained from all patients included in the study, which was approved by the local ethical committee.

Blood samples were obtained from all patients for the determination of anti-HCV antibodies, HCV viraemia, HBV markers, and HBV viraemia. Blood samples for HBV-DNA and HCV-RNA determination were taken before treatment, at 6 and 12 months after the beginning of the study, and when transaminase values were increased. Blood samples were centrifuged within 1 hour after withdrawal and stored at –80°C until further processing. Total DNA extraction from serum samples and HBV PCR were performed as described elsewhere [Zignego et al., 1994].

Serum HCV RNA was determined using a "one-step nested" PCR assay with primers corresponding to the 5′ noncoding region of the HCV genome as previously shown [Ferri et al., 1993]. For HCV genotyping, type-specific primers localized in the core region were used according to Okamoto et al. [1992] with minor differences [Zignego et al., 1996].

Anti-HBc of IgM class were tested using the IMx Core-M assay (Abbott Laboratories, North Chicago, IL) and semiquantitatively measured using reference standard curves. We considered an IMx value of 0.204 as a cutoff, which has been identified as the most sensitive and specific in chronic HBV infection (Colloredo Mels et al., 1993).

Anti-HCV antibodies were assayed in all patients by the RIBA II and enzyme-linked immunosorbent assay (ELISA) II (Chiron RIBA/ELISA HCV Second Generation Test System, Chiron Corp., Emeryville, CA; Ortho Diagnostic Systems, Raritan, NJ). All tests were carried out according to the manufacturer's instructions.

Statistical analysis was undertaken using Fisher's exact test.

RESULTS

The main virological characteristics of the 14 patients studied, including HCV genotype, are shown in Table II. Regarding the remaining 111 patients involved in the study, serum HCV-RNA was detected in 95 patients (86%) with genotypes 1a, 1b, 2 (a or c), or 3, detectable in nine (10%), 66 (68%), 17 (18%), and three (4%) patients, respectively.

By the end of the treatment period, serum ALT values had become normal in four of 14 patients (28%) (patients 3, 5, 9, and 10; Table I, II; Fig. 1). The remaining 10 patients had elevated ALT values, generally after a transient reduction during the first period of treatment (2–5 months) (Fig. 2). All patients completed the entire planned period of treatment (12 months) with the exception of three nonresponder patients, who stopped treatment after 6 months of therapy because of side effects or intolerance (patients 6, 11, and 14; Tables I, II). All responder patients (4/4) relapsed after therapy, with increased serum ALT levels, within 5 months of completion of therapy (range 2–5 months) (Fig. 1). Table II represents the pattern of HBV and HCV viraemia and the kinetics of IgM anti-HBc antibodies in serum before treatment, at time of ALT relapse during therapy or after therapy interruption, as well as at time of treatment withdrawal (12 or 6 months of IFN administration). Before therapy, all but one patient (patient 8; Table II) were both HBV and HCV PCR-positive, six of these (patients 1, 4, 5, 7, 10, and 11; Table II; Figs. 1, 2) were also both HBV-DNA- and HCV-RNA-positive during ALT elevations (nonresponders or relapsed responders during follow-up). In three patients (2, 3, and 5; Table II), only HCV-RNA was detected during ALT flare-up, whereas in the remaining five patients, including the patient who was HCV-RNA-negative before treatment (8, 9, 12, 13, and 14; Table II), only HBV-DNA was detected. All patients were persistently HBsAg-negative and anti-HCV-positive during the period of observation. Anti-HBc of IgM class were positive both before treatment and dur-

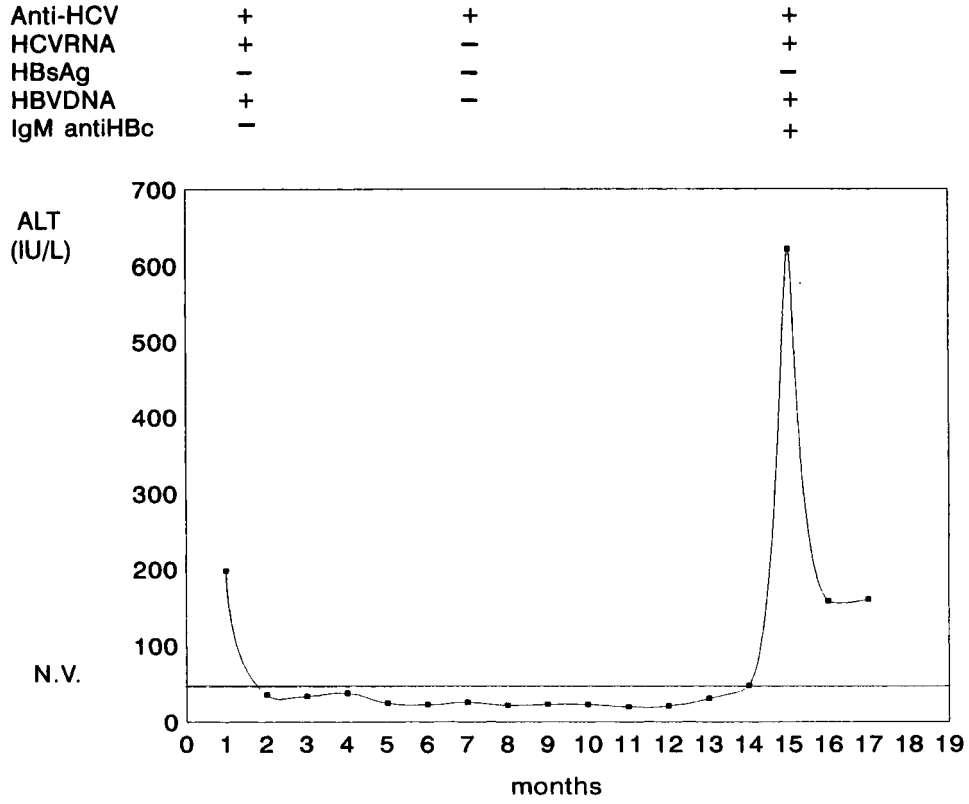

Fig. 1. Patterns of serum HCV and HBV markers (anti-HCV, HCV-RNA, HBsAg, HBV-DNA, IgM anti-HBc) in patient 10 who responded to IFN during treatment but relapsed during follow-up. N.V., ALT normal values.

ing ALT elevations in patients 8, 9, and 11 (Table II; Fig. 2) and only during the relapse of ALT values in patients 4, 10, and 12 (Table II; Fig. 1).

Among the remaining 111 anti-HCV-positive, HBsAg-negative, HBV-DNA (PCR)-negative patients who underwent the same therapeutical protocol, 18 (16%) stopped the treatment after 6–9 months because of side effects. *A* biochemical response to IFN treatment was observed in 60 cases (54%). There was a trend towards a lower initial response in HBV/HCV coinfected patients, but the difference was not significant ($P = 0.064$). Relapse of ALT values was observed in 28/60 cases (47%) during a follow-up of 1 year after treatment.

The sustained response observed in HBV-DNA-negative patients (32/111) was significantly higher than that shown in HBV-DNA-positive patients (0/14) ($P = 0.012$).

DISCUSSION

The effectiveness of α-IFN treatment was evaluated in HCV-positive chronic hepatitis patients with inapparent coinfection by HBV. It was found that less than one-third of patients presented an initial response and all "responder" patients relapsed after IFN withdrawal so that none had a sustained response. This suggests that an "inapparent" HBV coinfection in anti-HCV-positive patients may be implicated in cases of resistance to IFN treatment.

Several trials have shown that IFN therapy is the most promising approach for the control of chronic hepatitis C [Hoofnagle et al., 1987; Di Bisceglie et al., 1989; Alberti et al., 1992], even if IFN resistance (no response or relapse of ALT values after initial response) is a well-known and frequent event. The mechanisms of effectiveness as well as resistance to IFN are still unclear [Garson et al., 1995; Ounanian et al., 1995; Okada et al., 1992; Lau Jyn et al., 1993; Serfaty et al., 1994; Magrin et al., 1994; Pozzato et al., 1994; Zignego et al., 1992, 1995].

In preliminary studies, we observed that the presence of markers indicating past HBV infection (anti-HBV antibodies) was associated with a reduced rate of treatment response [Zignego et al., 1991]. However, this association was not statistically significant in a larger population (unpublished data), suggesting that anti-HBV antibody positivity may include two distinct groups of patients, some of whom were affected by an "inapparent" coinfection by HBV and HCV and others who resolved a past HBV infection and were infected only by HCV. In this study the patients had an HBV/HCV coinfection, which was "inapparent" by routine serological tests but was demonstrated by PCR. Interestingly, the majority of these coinfected subjects had anti-HBV antibodies.

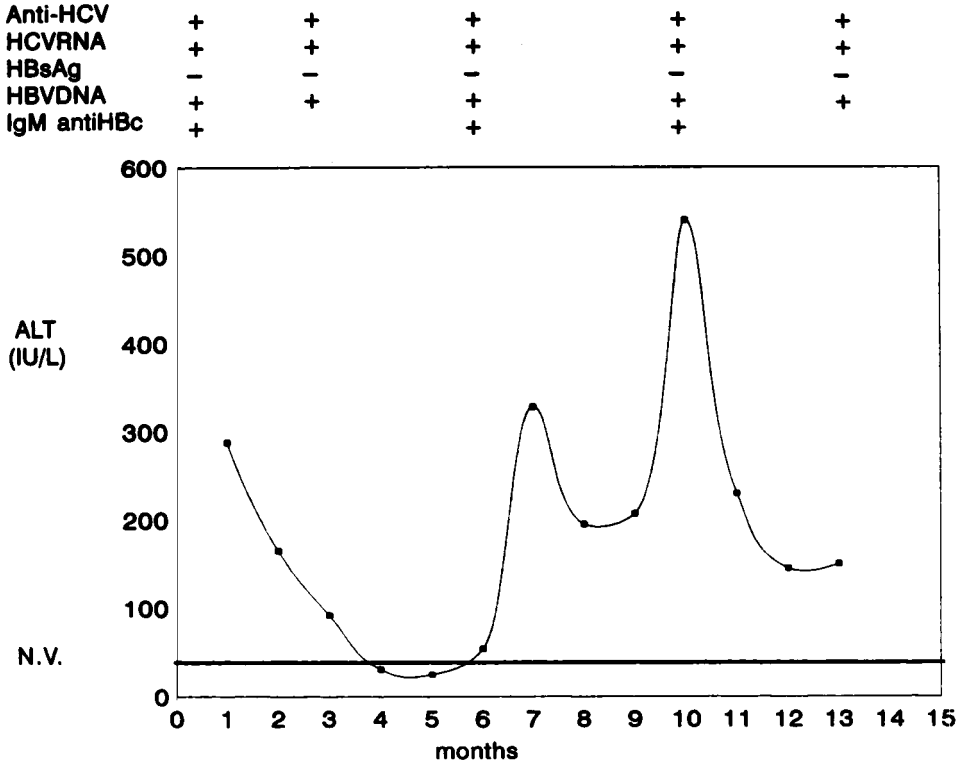

Fig. 2. Patterns of serum HCV and HBV markers (anti-HCV, HCV-RNA, HBsAg, HBV-DNA, IgM anti-HBc) in "nonresponder" patient 11. N.V., ALT normal values.

TABLE I. Main Clinical and Demographic Characteristics of Patients

Pt	Age (yr)	Sex	AST (nv 40 I/U)	ALT (nv 40 I/U)	Anti-HBs	Anti-HBc	Anti-ABe
1	40	M	80	147	–	–	–
2	45	M	138	212	–	+	–
3	39	M	80	85	+	+	–
4	48	M	117	224	+	+	–
5	63	M	147	80	+	–	–
6	59	M	98	108	–	–	–
7	50	M	121	292	–	+	+
8	52	M	151	286	+	+	–
9	33	F	110	137	+	+	+
10	48	M	117	198	+	+	+
11	60	F	242	367	+	–	–
12	50	M	169	480	+	+	–
13	56	M	206	298	–	+	–
14	50	M	126	251	+	+	+

Pt, patient's number; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

The percentage of persistent and sustained response in the group of patients with HBV/HCV coinfection was lower than that observed in control patients as well as those described in previous studies [Zignego et al., 1991; Hoofnagle et al., 1987; Di Bisceglie et al., 1989; Alberti et al., 1992]. In particular, patients with HCV/HBV coinfection showed a trend towards greater resistance to IFN treatment and a significant reduction of sustained response.

Additional information stems from the analysis of HCV/HBV viraemia kinetics during the treatment and at its withdrawal. In some patients, relapse of ALT values after IFN treatment was not associated with HCV or combined HCV/HBV viraemia but with an isolated HBV viraemia, in spite of persistent HBsAg negativity. The fact that higher doses of IFN are required for hepatitis B treatment than for HCV infection is in agreement with this hypothesis [Perillo, 1989; Fattovich et al., 1989] and suggests that IFN may have a selective role, thus favouring HBV persistence and replication in the patients studied.

Further data arise from the analysis of kinetics of anti-HBc antibodies of IgM class using a highly sensitive quantitative assay. Usually, the rise of IgM anti-

TABLE II. Anti-HBc Antibodies of IgM Class, HBV and HCV Viraemia (PCR) in Patients With Inapparent HBV Coinfection Before IFN Treatment, After the Time of ALT Relapse and at the End of Therapy Withdrawal and During Transaminase Flare Up

	Before treatment			ALT relapse				End of therapy (12 months)	
Pt	IgM anti-HBc	HBV-DNA PCR	HCV-RNA PCR*	IgM anti-HBc[a]	HBV-DNA PCR	HCV-RNA PCR	Months from start of therapy	HBV-DNA PCR	HCV-RNA PCR
1	– (0.071)	+	+(1b)	–(0.083)	+	+	2[b]	+	+
2	– (0.083)	+	+(1b)	–(0.120)	–	+	3[b]	–	+
3	–(0.104)	+	+(2)	–(0.151)	–	+	15[c]	–	–
4	– (0.073)	+	+(1b)	+(0.235)	+	+	2[b]	+	+
5	– (0.120)	+	+(1b)	–(0.151)	+	+	2[b]	+	+
6	– (0.151)	+	+(1b)	–(0.120)	–	+	6[d]	(d)	(d)
7	– (0.084)	+	+(1b)	–(0.101)	+	+	3[b]	+	+
8	+ (0.222)	+	–	+(0.230)	+	–	3[b]	+	–
9	+ (0.235)	+	+(1a)	+(0.266)	+	–	15[c]	–	–
10	– (0.158)	+	+(2)	+(0.287)	+	+	13[c]	–	n.d.
11	+ (0.381)	+	+(1b)	+(0.424)	+	+	6[d]	(d)	(d)
12	– (0.158)	+	+(1b)	+(0.268)	+	–	3[b]	+	–
13	– (0.123)	+	+(1b)	–(0.170)	+	–	2[b]	+	–
14	– (0.153)	+	+(1b)	–(0.160)	+	–	6[d]	(d)	(d)

HB = for a correct interpretation of the corrections made, please see the enclosed, modified Table II.
* = HCV genotype is given in brackets.
[a]IgM anti-HBc = IMx.
[b]Time of ALT relapse in patients with transient response during IFN treatment.
[c]Time of ALT relapse in patient responders at the 12th month of therapy (15 and 13 = third and first month of follow-up, respectively).
[d]Patients who did not experience normalisation of ALT values during IFN treatment and who stopped treatment at six months.
Pt, patient number; n.d., not done; PCR, polymerase chain reaction; IFN, interferon; ALT, alanine aminotransferase.

HBc is reported to be correlated with HBV-induced liver damage and consequently with ALT flare-ups [Gerlich et al., 1986; Brunetto et al., 1988, 1993; Colloredo Mels et al., 1993, 1994; Marinos et al., 1994]. In the present study, IgM anti-HBc was found in some patients in association with HBV-DNA positivity both before treatment and during relapse. This may confirm the supposed pathogenetic role played by HBV in the observed liver disease, in spite of its low replicative levels. In contrast, the appearance of these antibodies only after IFN treatment in other patients seems to confirm the hypothesis that ineffective IFN doses had a selective activity on HBV replication. Finally, negative results observed in some patients before treatment or during ALT flare-ups might be attributed to viral interference between the two viruses with an imbalance towards HCV [Pontisso et al., 1993, 1994] or to an early sample drawing, respectively.

In conclusion, this study suggests that an "inapparent" HBV coinfection in anti-HCV-positive CLD patients may be an additional negative prognostic factor influencing the behaviour of IFN treatment. Further studies will show the effects of different treatment schedules in these patients. HBV-DNA and/or IgM anti-HBc detection by very sensitive methods may be important both as prognostic factors and as a tool for better understanding intervirus relationships and mechanisms involved in multiple hepatitis virus infections.

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